

IN THE CLAIMS:

Amend the claims as follows.

Claims 1-106. (Canceled)

107. (Currently Amended) A method for producing a luciferase which is substantially free of enzymatically active *E.coli* adenylate kinase, the method comprising culturing an *E. coli* host cell which has been transformed so that it expresses a luciferase which is thermostable at 37°C, and expresses adenylate kinase only in a mutant form which form is denatured at a temperature ~~temperatures~~ of 37°C; and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to a temperature at which the adenylate kinase is denatured but the luciferase remains substantially unaffected, so as to denature the adenylate kinase.

108. (Currently Amended) A method according to claim 107 wherein the luciferase is a luciferase selected from the group consisting of Photinus pyralis luciferase which has a mutation at position 354 in the amino acid sequence, and a ~~a or a~~ *Luciola* luciferase with a mutation at position 354, which mutation elevates the thermostability of the protein over that of the wild-type protein.

109. (Currently Amended) A method according to claim 107 wherein the luciferase is a selected from the group consisting of *Luciola* luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid.

110. (Previously Presented) A method according to claim 107 wherein the *E. coli* host cells are cultured for a period which is sufficient to allow production of the luciferase, and then a batch of said culture is subjected to a temperature at which the adenylate kinase is denatured, and the luciferase is recovered from the said batch.

111. (Currently Amended) A method according to claim 107 wherein the adenylate kinase comprises a mutation at amino acid ~~includes mutations at amino acids~~ 87 or 107 in the sequence of *E. coli* adenylate kinase.

112. (Currently Amended) A method according to claim ~~106~~ 107 wherein the said temperature is a temperature of from 37°C up to a temperature below the ~~temperature at which the luciferase is denatured.~~

113. (Previously Presented) A recombinant *E. coli* cell which has been transformed so that it expresses a first nucleotide sequence which encodes a luciferase which is stable at 37°C under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate kinase only in a mutated form which is denatured at 37°C.

114. (Previously Presented) A recombinant cell according to claim 113 which further comprises at least one selection marker.

115. (Previously Presented) A recombinant cell according to claim 113 wherein the luciferase is a Photinus pyralis luciferase which has a mutation at position 354 in the amino acid sequence, or a *Luciola* luciferase with a mutation at position 354, which mutation elevates the thermostability of the protein over that of the wild-type protein.

116. (Previously Presented) A recombinant cell according to claim 113 wherein the luciferase is a *Luciola* luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid.

117. (Currently Amended) A method for producing a recombinant cell according to claim 113 which method comprises in any order (a) transforming a host cell with a vector which encodes adenylate kinase in a form which is denatured at 37°C, subjecting transformants to a temperature of 37°C or more ~~said conditions~~ and detecting those in which do not grow as a result of lack of active adenylate kinase ~~protein-product is denatured~~, and (b) transforming said host cell with a vector which encodes the said luciferase and a first selection marker, and using the first selection marker to detect stable transformants.

118. (Previously Presented) A method according to claim 117 wherein the vector which encodes said adenylate kinase further comprises a second selection marker which is different to said first selection marker, and stable transformants are selected.

119. (Currently Amended) A method according to claim 118 ~~116~~ wherein said selection markers comprise ~~particular~~ different antibiotic resistance genes.

120. (Currently Amended) A method for producing a luciferase which is substantially free of enzymatically active *E.coli* adenylate kinase, the method comprising culturing an *E. coli* host cell which has been transformed so that it expresses a luciferase selected from the group consisting of Photinus pyralis luciferase which has a mutation at position 354 in the amino acid sequence, and a ~~or a~~ *Luciola* luciferase with a mutation at position 354, which mutation elevates the thermostability of the protein over that of the wild-type protein, and expresses adenylate kinase only in a mutant form which comprises a mutation at amino acid ~~has mutations at amino acids~~ 87 or 107 in the sequence of *E. coli* adenylate kinase; and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to a temperature at which the adenylate kinase is denatured but the luciferase remains substantially unaffected, so as to denature the adenylate kinase.

121. (Previously Presented) A method according to claim 120 wherein the *E. coli* host cells are cultured for a period which is sufficient to allow production of the

luciferase, and then a batch of said culture is subjected to a temperature at which the adenylate kinase is denatured, and the luciferase is recovered from the said batch.

122. (Currently Amended) A method according to claim 121 wherein the said temperature is a temperature of from 37°C up to a temperature below the temperature at which the luciferase is denatured.

123. (Currently Amended) A recombinant *E. coli* cell which has been transformed so that it expresses a luciferase selected from the group consisting of Photinus pyralis luciferase which has a mutation at position 354 in the amino acid sequence, and a ~~or a~~ Luciola luciferase with a mutation at position 354, which mutation elevates the thermostability of the protein over that of the wild-type protein, under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate kinase only in a mutated form which has mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase.

124. (Previously Presented) A recombinant cell according to claim 123 which further comprises at least one selection marker.

125. (Currently Amended) A method for producing a recombinant cell according to claim 123 which method comprises in any order (a) transforming a host cell with a vector which encodes adenylate kinase in a form which comprises a mutation at amino acid ~~has mutations at amino acids~~ 87 or 107 in the sequence of *E. coli* adenylate kinase,

subjecting transformants to a temperature of 37°C or more ~~said conditions and~~
detecting those ~~in which~~ do not grow as a result of lack of active adenylate kinase
~~protein product is denatured~~, and (b) transforming said host cell with a vector which
encodes the said luciferase and a first selection marker, and using the first selection
marker to detect stable transformants.

126. (Previously Presented) A method according to claim 125 wherein the vector
which encodes said adenylate kinase further comprises a second selection marker
which is different to said first selection marker, and stable transformants are selected.

127. (Previously Presented) A method according to claim 126 wherein said
selection markers comprise particular different antibiotic resistance genes.

128. (Currently Amended) A method for producing a luciferase which is
substantially free of enzymatically active *E.coli* adenylate kinase, the method comprising
culturing an *E. coli* host cell which has been transformed so that it expresses a *Luciola*
luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino
acid, and expresses adenylate kinase ~~and expresses adenylate kinase only in a mutant~~
form which comprises a mutation at amino acid ~~has mutations at amino acids 87 or 107~~
in the sequence of *E. coli* adenylate kinase; and recovering the luciferase, wherein
either the host cell culture or the recovered luciferase is subjected for a sufficient period
of time to a temperature at which the adenylate kinase is denatured but the luciferase
remains substantially unaffected, so as to denature the adenylate kinase.

129. (Previously Presented) A method according to claim 128 wherein the *E. coli* host cells are cultured for a period which is sufficient to allow production of the luciferase, and then a batch of said culture is subjected to a temperature at which the adenylate kinase is denatured, and the luciferase is recovered from the said batch.

130. (Currently Amended) A method according to claim 128 wherein the said temperature is a temperature of from 37°C up to a temperature below ~~the temperature~~ at which the luciferase is denatured.

131. (Currently Amended) A recombinant *E. coli* cell which has been transformed so that it expresses a *Luciola* luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid, under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate kinase only in a mutated form and expresses adenylate kinase only in a mutant form which comprises a mutation at amino acid ~~has mutations at amino acids~~ 87 or 107 in the sequence of *E. coli* adenylate kinase.

132. (Previously Presented) A recombinant cell according to claim 131 which further comprises at least one selection marker.

133. (Currently Amended) A method for producing a recombinant cell according to claim 131 which method comprises in any order (a) transforming a host cell with a

vector which encodes adenylate kinase in a form which comprises a mutation at amino acid ~~has mutations at amino acids~~ 87 or 107 in the sequence of *E. coli* adenylate kinase, subjecting transformants to a temperature of 37°C or more ~~said conditions~~ and detecting those ~~in which~~ do not grow as a result of lack of active adenylate kinase ~~protein product is denatured~~, and (b) transforming said host cell with a vector which encodes the said luciferase and a first selection marker, and using the first selection marker to detect stable transformants.

134. (Currently Amended) A method according to claim 133 ~~131~~ wherein the vector which encodes said adenylate kinase further comprises a second selection marker which is different to said first selection marker, and stable transformants are selected.

135. (Currently Amended) A method according to claim 134 wherein said selection markers comprise ~~particular~~ different antibiotic resistance genes.